1999

Report of the *Brassica* Crops Working Group

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INTRODUCTION

The Brassicaceae family comprises about 3000 species, the majority of which are found in the Northern Hemisphere. Many common agricultural weeds, such as *Brassica nigra* (L.) Koch, *Brassica rapa* L., *Cardaria draba* (L.) Desv., *Raphanus raphanistrum* L., and *Sinapis arvensis* L., belong to this family. The most important crop species from this family are the oilseed Brassicas; *Brassica napus* L., *B rapa* L., and *B juncea* Coss., which are generally referred to as rapeseed, oilseed rape, or canola. Other widely cultivated species in this family are: *B. oleracea* L. (cabbage, kale, kohlrabi, Brussels sprouts, cauliflower, and broccoli), *B. chinensis* L. (syn. B. *napus* var. *chinensis*; Chinese cabbage), *Raphanus sativus* L. (radish), and *Armoracia rusticana* Gaertn. (horseradish). We agreed to limit our discussion to the rapeseed species because they are the focus of most of the gene transfer technology. Worldwide, rapeseed is grown on more than 20 million hectares; it is the third most important oil plant after palm oil and soybean. Major producers are China, India, Canada, the European Community, and Australia.

Reproductive Biology

Brassica napus can be self-pollinated or cross-pollinated. In cultivated fields, cross-pollination rates of about 20-30% have been reported (Rakow and Woods 1987). The frequency of cross-pollination is influenced by weather, availability of insect pollinators, and cultivar. *Brassica rapa* varieties are generally self-incompatible. Both species are primarily pollinated by honeybees (Williams *et al*. 1987), however wind pollination is possible over distances of up to 2.5 km (Timmons *et al*. 1995). *B. rapa* is a diploid with chromosome number of 2n=20, and *B. napus* is an allotetraploid with a chromosome number of 2n=38 (Hosaka *et al*. 1990).

Hybridization

Crosses between *B. napus* and other related species occur, but the rate of success varies depending on the species. The following spontaneous (without emasculation or manual pollination) hybridizations have been documented: *B. napus* × *B. rapa*, *B. juncea* × *B. napus*, *B. nigra* × *B. napus*, *B. napus* × *Hirschfeldia incana*, and *B. napus* × *Raphanus raphanistrum*. In all of the above hybridizations, F_2's and backcross progeny were produced (OECD 1997).

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

Seed Dormancy

Seed dormancy in crop plants is generally undesirable because dormant seeds may germinate at inappropriate times, and because additional seed is required to compensate for the proportion that will not germinate. Breeders of *B. rapa* have not yet succeeded in removing this weedy trait completely. Seed dormancy is much less of a problem in *B. napus*, whose mature seed has virtually no primary dormancy (Lutman 1993; Schlink 1994). It is possible however, that *B. napus* seed can acquire secondary dormancy and remain viable in the soil for at least 5 years (Schlink 1994). Secondary dormancy can be induced in the absence of light when available moisture is insufficient for germination, conditions that may occur when seeds are incorporated into the soil after harvest (Pekrun *et al*. 1997; Pekrun *et al*. 1998).

In weedy relatives of rapeseed, seed dormancy is a very powerful survival mechanism. Dormant seeds may germinate over a period of several weeks during the growing season, which greatly improves the chance that at least some plants will be successful in replenishing the seedbank. The presence of flowering individuals during a large portion of the growing season increases the opportunity for cross-pollination with populations that produce only one cohort per season. Furthermore, seed dormancy may allow a plant population to survive one or several seasons of complete reproductive failure. Whether or not a particular hybrid will exhibit seed dormancy is rather unpredictable (Landbo and Jørgensen 1997).

Novel Resistance Traits

Within the last ten years, numerous novel traits have been genetically engineered into the *Brassica* genome, however, only a relatively small number have found their way into commercial varieties. Some of these traits were introduced to confer resistance to herbicides, insects, and disease organisms. The herbicide resistance traits were particularly successful because they provided farmers with new and much needed weed control options. Plants with engineered disease resistance genes have not yet been released commercially, but field trials are underway. The table below summarizes transgenic resistance traits in rapeseed varieties that are commercially available or currently being field-tested.

Table 1. Novel traits introduced into *B. napus* and/or *B. rapa* varieties.

Phenotype	Transgene(s)
Resistance to glufosinate-ammonium	Phosphinothricin acetyltransferase (PAT) gene from *Streptomyces hygroscopicus*
Resistance to glyphosate	Roundup-Ready™ gene
Resistance to imidazolinone (imazethapyr)	Acetohydroxyacid synthase (AHAS) gene from *Arabidopsis*
Resistance to chlorosulfuron	Acetohydroxyacid synthase (AHAS) gene from *Arabidopsis*
Resistance to Turnip Yellow Mosaic Virus	Noncoding regions of TYMV genomic RNA
Resistance to fungal infection	Chitinase
Insect resistance	Synthesis of *Bacillus thuringiensis* insecticidal crystal protein (Bt cryIAc)

EVIDENCE OF INTROGRESSION OF PEST RESISTANCE GENES

Pest resistance genes are bred into cultivated varieties to prevent yield loss. In agricultural systems, pest resistant varieties generally have a significant advantage over non-resistant varieties, and this advantage typically translates into a rapid spread of the resistant variety at the expense of the non-resistant varieties. Humans mediate the spread of pest resistant crop varieties. Whether introgression of a pest resistant gene into a feral population would increase the spread of a wild population is open to question. We, as a group, were not aware of any example where introgression of a pest resistance gene, or the consequences of such an event, had actually been documented.

There is, however, ample evidence that hybridization between closely related species occurs spontaneously (OECD 1997); introgression of resistance genes is therefore quite plausible. Available commercial varieties already have varying degrees of resistance to two of the most common fungal pathogens, *Leptosphaeria maculans* and *Albugo candida*. These traits, identified in related germplasm, were introduced into cultivars by conventional breeding. It is reasonable to assume that these resistance traits already exist within the genome of the closely related *Brassica* species complex, so their reintroduction via gene flow from cultivated transgenic varieties is not likely to have much influence on the fitness of feral populations.

The situation may be different with respect to many of the engineered resistance traits likely to be available over the next few years. Such new traits, imparted for example by a set of stacked genes that confer broad insect and fungus resistance, may protect plants from a wide range of pests. It is conceivable that this situation could trigger an increase in the size and range of the population in question.

There is little doubt that genes from transgenic rapeseed have the potential to escape into related varieties and species. Chèvre *et al.* (1997) documented the introgression of glufosinate ammonium resistance from B. *napus* to *Raphanus raphanistrum* under experimental conditions. Spontaneous production of crop-weed hybrid seeds under field conditions was reported from Denmark and the Netherlands (Jørgensen *et al.* 1996; De Vries *et al.* 1992). Consequently, the escape of transgenes is certainly a cause for concern.

Data from a field experiment with *Brassica napus* containing a *Bacillus thuringiensis* cry1Ac transgene suggest that this pest resistance gene is not likely to have a significant impact on weediness (Stewart *et al.* 1997). The Bt gene conferred increased fitness under moderate selection pressure by *Plutella xylostella*, however, this did not translate into increased competitiveness, nor did the transgenic plants exhibit greater weediness (Stewart *et al.* 1997).

IDENTIFICATION OF KNOWLEDGE GAPS

During the plenary session of this workshop, it was argued that scientists have numerous questions they would *like* to have answered, but what *really* needs to be known is a much more restricted set of information. We partially disagree with this statement because essential knowledge can only be identified once we have a sufficient general understanding of the system under consideration.

Our current knowledge tells us that pest resistant transgenic rapeseed varieties are likely to hybridize with a number of weedy species (OECD 1997) and that there is a good chance that transgenic traits will eventually introgress into populations of weedy species (Mikkelsen *et al.* 1996; Metz *et al.* 1997). We know from ecological work that herbivory by insects and other organisms is the principal factor in many environments that limits the abundance of plant species (Louda and Potvin 1995). Hence, there is a definite possibility that the newly acquired pest resistance will result in greater fitness of weedy *Brassica* species, which could be expected to become more abundant. Such an event could threaten biodiversity by displacing other plant species as well as their associated fauna. From a strictly agronomic standpoint, the increased abundance of a weed is also likely to be undesirable.

It may therefore be argued that transgenic pest resistant rapeseed varieties should not be released because potential negative effects due to the spread of pest resistance genes are unacceptable. However, before making such a decision we should also consider potential positive effects of this technology, including the health, environmental, and economic benefits due to a reduction in pesticide use. In order to weigh the positive effects against the negative, a more detailed analysis is required.

Current knowledge of the mechanisms that determine population dynamics of a weedy species is at best sketchy, even for the most extensively studied weeds. We have little quantitative information on the influence of insects, pathogens, and other organisms on the relative abundance of weeds. Data from

ecological research, as well as from biocontrol studies, show that reductions in population growth caused by insects and/or pathogens are often dependent on environmental factors (Louda and Rodman 1996). A geographic information system that matches the environment with pest and host species would provide valuable information about the potential impact of increased pest resistance. Introgression of a pest resistance gene has the potential to increase plant fitness only in areas where the pest is present and where environmental conditions are favorable to the pest.

We also need to know how frequently introgression would occur, how quickly such populations would spread, and the fate of the gene within the population. The frequency of hybridization events and subsequent introgression may depend on the time of flowering, presence of pollination vectors, and degree of sexual compatibility. Spread at the landscape level will largely depend on seed dispersal mechanisms. Seeds that rely on wind, water, animals, or humans for dispersal may travel long distances and spread their genes much faster than seeds that are only locally dispersed. Introgression and dispersal can easily be simulated, but actual data is needed to parameterize the model and to test underlying hypotheses.

Information on the frequency of hybridization events and on the rate at which pest resistance genes may spread could be obtained with the use of marker genes. Furthermore, such genes could help reduce the risk associated with gene escape by facilitating identification of hybrid individuals. The green fluorescent protein (GFP) gene is a commonly used transgenic marker that could easily be inserted along with a pest resistance gene (Stewart 1996).

A complete record of wild species that have the potential to hybridize with the rapeseed crops is needed. We should also be concerned with compatible species outside the US because transgenic varieties will eventually cross national, and therefore regulatory, boundaries. Furthermore, we need some measure of the likelihood of specific hybridization events and the level of fertility of subsequent generations.

Questions also remain on how transgenes are expressed when moved from one species to another. Current evidence shows that transgenes are expressed similarly in hybrids and parent plants (Chèvre *et al*. 1997; Stewart *et al*. *in press*). It is, however, conceivable that situations could exist in which a pest resistance gene may not function as expected when transferred to a different host. Unexpected behavior must be taken into consideration in order to evaluate the risks properly.

Filling these knowledge gaps should allow us to build simulation models that can quantify the impact of introgression of pest resistance traits on fitness and abundance of sexually compatible species. Based on this information it would be possible to make a more accurate risk assessment that would consider costs as well as benefits of a proposed technology.

RESEARCH NEEDS

Because of the above knowledge gaps, we recommend support for the following kinds of research projects:

Creation of a Database of Sexually Compatible Species and Varieties.

A database should be created to provide users with an exhaustive list of sexually compatible species, information on their geographic distribution, time of flowering by geographic region, details about hybridization success, and an exhaustive list of pests. This project would require the collaboration of numerous institutions and individuals from a wide range of disciplines. The database should be made accessible to the public, preferably over the internet, and would need to be updated as new information becomes available. Much of the information could be obtained from existing sources, but funds should be made available to conduct hybridization studies.

Development of Geographic Information System of Pest Influence.

This geographic database would combine distribution maps of compatible species with distribution maps of pest species and a range of layers for environmental variables. The objective would be to produce a map that indicates the

expected impact of a pest organism on a given host. The stronger the impact, the greater would be the effect of a corresponding pest resistance gene.

Creation of Long-Term Studies.

Long-term studies are needed to monitor weed populations for changes in gene frequencies and to determine the influence of such changes on pest populations. Far too few of these studies exist, especially in North America. The data would be very useful in validating simulation models of population genetics.

Exclusion Studies to Measure the Influence of Pest Pressure on Reproductive Rates.

Well-designed exclusion studies can explain the influence of specific pest populations on the fitness of plant populations. Such studies should be conducted over a range of environments, and critical environmental variables measured. Levels of pest pressure can be varied by adjusting pesticide application rates, as would occur with partial resistance. These kinds of data are required to develop and parameterize models that can simulate the influence of pest pressure on reproductive rates.

Hybridization and Introgression Experiments.

Hybridization and introgression experiments should be conducted with the most relevant compatible species and include follow-up studies to measure the persistence and performance of the transgene in the environment. In addition to using single pest resistance genes, these experiments may also be conducted with stacked genes that include herbicide resistance.

Observational Studies of Basic Reproductive Biology.

Observational studies describing the reproductive biology of the lesser known related species are needed. Such studies should provide information about the time of flowering, degree of outcrossing, principal pollination vectors, reproductive mechanisms that isolate the species, seed dispersal, and seed survival.

Modeling Projects.

Simulation models are needed to synthesize available knowledge and to direct further research. Risk assessment will have to be conducted on simulated outcomes. The quality of the risk assessment can be expected to be in proportion to the quality of the simulation model used.

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